Establishment of the WHO 1st International Standard ADAMTS13, plasma (12/252)

OFFICIAL COMMUNICATION OF THE SSC

A R HUBBARD,* A B HEATH* and J A KREMER HOVINGA† ON BEHALF OF THE SSC SUB-COMMITTEE ON VON WILLEBRAND FACTOR OF ISTH

**National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK; †University Clinic of Hematology and Central Hematology Laboratory, Bern University Hospital and the University of Bern, Switzerland.*

Correspondence:
Dr A R Hubbard
Haemostasis Section
Biotherapeutics Group
National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Potters Bar
Hertfordshire
EN6 3QG
United Kingdom

Tel: +44 (0) 1707 641318 Fax: +44 (0) 1707 641050

E-mail: anthony.hubbard@nibsc.org

Running title: Standardisation of ADAMTS13 in plasma

Word count text: 1474

ADAMTS13 (**A** **D**isintegrin **A**nd **M**etalloprotease with **T**hrombo**S**pondin type 1 motifs **13**), also known as "von Willebrand factor (VWF) cleaving protease" is responsible for modulating the size of VWF multimers in the circulation. Acquired or congenital deficiency of ADAMTS13 is associated with the circulation of ultra-large multimers of VWF which can lead to thrombotic thrombocytopoenic purpura (TTP) characterised by disseminated platelet aggregation and thrombosis in the microcirculation, severe platelet deficiency, red cell haemolysis and organ damage [1,2]. Measurement of ADAMTS13 activity in plasma is an important component in the diagnosis and treatment of TTP and numerous methods, both commercial and "in house", are available for the estimation of ADAMTS13 activity and antigen [3]. However, there is currently no internationally accepted unitage to support harmonisation of measurement between laboratories hence the development of the World Health Organisation 1st International Standard (WHO IS) for ADAMTS13 in plasma. The candidate WHO IS (coded 12/252) was prepared from a pool of 38 donations from normal healthy donors and 1 ml aliquots were dispensed into approximately 10,000 glass ampoules prior to freeze-drying and sealing.

A collaborative study involving 32 laboratories from 14 countries was undertaken to assign values for activity and antigen to the candidate WHO IS based on assays relative to local pooled normal plasma preparations. The candidate WHO IS was included in the study as coded duplicates (samples A & B). Most laboratories used VWF A2 domain peptide substrate assays either in a Fluorescence Resonance Energy Transfer (FRET) assay (n=18) or an activity ELISA (n=9) to measure ADAMTS13 activity and all laboratories used ELISA for antigen measurement. Comparison of the candidate WHO IS with the local normal pools was associated with a high degree of validity in terms of parallelism of the dose-response relationships with only 9/117 activity assays and 8/58 antigen assays excluded because of non-parallelism. There was no significant difference between the coded duplicates of the

candidate WHO IS for estimates of activity or antigen nor between estimates of activity using the FRET and the activity ELISA methods. Combination of all results for activity gave an overall mean of 0.91 units/ml for the candidate WHO IS with inter-laboratory variability (geometric coefficient of variation, GCV) of 12.4%. For estimates of ADAMTS13 antigen the combination of all results gave an overall mean of 0.92 units/ml with inter-laboratory variability (GCV) of 16.3%.

Two plasma exchange waste bag samples (coded C & D) from a patient with acquired ADAMTS13 deficiency due to an inhibitory autoantibody, treated with plasma exchange, were also included in the study. The level of ADAMTS13 in sample C was below the limit of detection for assays of activity in many cases (21/32) and calculated estimates were only possible in 11 laboratories. However, 31/32 results were consistent with a severe deficiency below 0.1 units/ml. Patient sample D contained a higher level of ADAMTS13 and 24/32 data sets from the activity assays could be quantified. The overall mean estimate for activity in sample D was 0.15 units/ml with 23/24 laboratories agreeing levels below 0.3 units/ml whereas the mean estimate for antigen was higher at 0.63 units/ml (Fig 1). Estimates of both activity and antigen in sample D, calculated relative to the local pools, were associated with large inter-laboratory variability (GCVs 54% and 46% respectively) and there was little or no improvement when estimates were calculated relative to the proposed WHO IS (GCVs 63% and 32% respectively). This indicates that the large inter-laboratory variability is not primarily caused by the use of different local plasma pools but may also include methodological differences between laboratories as previously suggested in reports from other multi-centre studies, particularly when assaying samples with acquired ADAMTS13 deficiency [4,5,6]. The availability of a common reference material (WHO IS) should facilitate future work to identify the causes of inter-laboratory variability. Ratios of mean activity to antigen for patient samples C and D were greatly reduced at 0.11 and 0.24

respectively compared to normal plasma (0.99). This finding is probably related to the presence of circulating ADAMTS13-antibody complexes where ADAMTS13 activity is inhibited but which can still be detected by assays for antigen [7]. Assays of recombinant ADAMTS13 (sample E) indicated valid comparison of dose-response relationships with normal plasma (proposed WHO IS) but large inter-laboratory variability of estimates for both activity (GCV 45%) and antigen (GCV 72%). This variability could reflect the high dilution factors required for the assay of the recombinant sample or indicate that the proposed WHO IS is not optimal for measuring recombinant ADAMTS13.

The proposal to assign the consensus mean values for ADAMTS13 activity (0.91 IU per ampoule) and antigen (0.92 IU per ampoule) to the WHO 1st IS ADAMTS13 Plasma (12/252) was endorsed by the Expert Committee on Biological Standardisation of WHO in October 2014 [8].

Addendum

A Hubbard and J Kremer Hovinga were responsible for preparing the test samples and managing the multi-centre collaborative study. Statistical analysis and assay design were provided by A Heath.

Acknowledgements

We are very grateful to the participants in the collaborative study and to the chair and members of the ISTH/SSC Sub-committee for von Willebrand Factor. We also acknowledge Baxter Innovations GmbH, Vienna, Austria for the supply of recombinant ADAMTS13 and Kainos Laboratories, Tokyo, Japan for test kits used in the evaluation of the candidate materials.

Collaborative Study Participants

C Caron, Lab d'Hématologie, Centre de Biologie Pathologie, CHR, Lille, France

HCJ Eikenboom, University Medical Center, Leiden, The Netherlands

S Maitzen / M Blum, Baxter AG, Orth/Donau, Austria

C Eckmann / J Rentenaar, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

S Haberichter, Blood Center of Wisconsin, Milwaukee WI, USA

JA Kremer Hovinga, Bern University Hospital, Bern, Switzerland

J Owen, Wake Forest School of Medicine, Winston-Salem NC, USA

S Neelamegham, State University of New York, Buffalo NY, USA

Y Fujimura, Nara Medical University, Kashihara City, Nara, Japan

T Falter, University Medical Centre, Mainz, Germany

M Mirabet, Biokit R&D, Barcelona, Spain

S Kato, Alfresa Pharma Corporation, Ibaraki-City, Osaka, Japan

M Diaz-Ricart, Hospital Clinic, Barcelona, Spain

IJ Mackie, Dept. Haematology, University College London, London, UK

A Bonnefoy, CHU Saint-Justine, University of Montreal, Montreal, Quebec, Canada

JCM Meijers, Academic Medical Center, Amsterdam, The Netherlands

AM Lombardi, Department of Medicine, University of Padova, Padova, Italy

JE Sadler / J Muia, Washington University School of Medicine, St Louis MO, USA

C Kimchi-Sarfaty, OBRR / CBER / FDA, Bethesda MD, USA

AK Enjeti / K Chapman, John Hunter Hospital, New Lambton NSW, Australia

R Baker, Centre for Thrombosis & Haemophilia, Murdoch University, Perth, Australia

A Hubbard / S Daniels, Haemostasis Section, NIBSC, Potters Bar, UK

A Veyradier, Dept Hematology, Hôpital Antoine Béclère, Clamart, France

S Voisin, Lab d'Hématologie, Hôpital Rangueil, Toulouse, France

E Grandone / G Tiscia, Casa Sollievo della Sofferenza Institute, San Giovanni Rotondo, Italy

U Budde, Hämostaseology, Asklepios Klinik Altona, Hamburg, Germany

P Turecek / H Rottensteiner, Baxter Innovations GmbH, Vienna, Austria

F Peyvandi / C Valsecchi, ABB Haemophilia & Thrombosis Center, Milan, Italy

N Binder, Technoclone GmbH, Vienna, Austria

S Kitchen / RE Fretwell, Coagulation Dept., Royal Hallamshire Hospital, Sheffield, UK

SC Nair, Div. Haematology, Christian Medical College, Vellore, Tamil Nadu, India

K Vanhoorelbeke, Lab Thrombosis Research, IRF Life Sciences, Kortrijk, Belgium

References

1 Sadler JE. Von willebrand factor, ADAMTS-13, and thrombotic thrombocytopoenic purpura. *Blood* 2008; **112**: 11-18.

2 George JN, Nester CM. Syndromes of thrombotic microangiopathy. *New Engl J Med* 2014; **371**: 654-66.

3 Peyvandi F, Palla R, Lotta LA, Mackie I, Scully MA, Machin SJ. ADAMTS-13 assays in thrombotic thrombocytopoenic purpura. *J Thromb Haemost* 2010; **8**: 631-40.

4 Studt J-D, Böhm M, Budde U, Girma J-P, Varadi K, Lämmle B. Measurement of von Willebrand factor-cleaving protease (ADAMTS13) activity in plasma: a multicenter comparison of different assay methods. *J Thromb Haemost* 2003; **1**: 1882-7.

5 Tripodi A, Chantarangkul V, Böhm M, Budde U, Fong J-F, Friedman KD, Galbusera M, Girma J-P, Moake J, Rick ME, Studt J-D, Turecek PL, Mannucci PM. Measurment of von Willebrand factor cleaving protease (ADAMTS-13): results of an international collaborative study involving 11 methods testing the same set of coded plasmas. *J Thromb Haemost* 2004; **2**: 160-9.

6 Tripodi A, Peyvandi F, Chantarangkul V, Palla R, Afrasiabi A, Canciani MT, Chung DW, Ferrari S, Fujimura Y, Karimi M, Kokame K, Kremer Hovinga JA, Lämmle B, De Meyer SF, Plaimauer B, Vanhoorelbeke K, Varadi K, Mannucci PM. Second international collaborative study evaluating performance characteristics of methods measuring the von Willebrand factor cleaving protease (ADAMTS-13). *J Thromb Haemost* 2008; **6**: 1534-41.

7 Rieger M, Ferrari S, Kremer Hovinga JA, Konetschny C, Herzog A, Koller L, Weber A, Remuzzi G, Dockal M, Plaimauer B, Scheiflinger F. Relation between ADAMTS13 activity and ADAMTS13 antigen levels in healthy donors and patients with thrombotic microangiopathies (TMA). *Thromb Haemost* 2006; **95**: 212-20.

8 Hubbard AR, Heath AB, Kremer Hovinga JA. Value assignment of the proposed WHO 1st International Standard ADAMTS13, plasma (12/252). WHO/BS/2014.2246 World Health Organization Expert Committee on Biological Standardization 2014. http://www.who.int/biologicals/BS_2246_IS_ADAMTS13.pdf?ua=1

Figure 1 Mean laboratory estimates for ADAMTS13 activity and antigen in patient plasma (sample D) relative to the local normal plasma pools (L) and the WHO 1st IS ADAMTS13 Plasma (12/252) (IS). Laboratory estimates below the level of quantification are plotted as zero